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Dietary soy isoflavones during pregnancy suppressed the immune function in male offspring albino rats

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ABSTRACT

Phytoestrogens have an impact on both animals and humans due to use of legumes in animal diets as well as the increase of vegetarian diets in some human populations. Phytoestrogens thought to have varieties of adverse effects, among which immune system was involved. The present study aimed to investigate the effect of prenatal exposure to dietary soy isoflavones on some immunological parameters in male albino rat offspring. The pregnant rats were divided to three groups (12/group). Control group (free soy isoflavones), low soy isoflavones group (6.5%) and high soy isoflavones group (26%). The male offspring cell-mediated immune response was determined using phytohemagglutinin (PHA) injection and the intumesce index which was calculated on postnatal day 50 (PND 50). At PND 50, blood samples were collected for interleukin 12 (IL-12), interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) determination. Spleen, thymus, and PHA injected footpads were fixed for histopathology. Intumesce index, IL-12, IFN-γ, spleen and thymus relative weights were significantly ($P < 0.05$) decreased in offspring born to dams fed low and high dietary soy isoflavones. In contrary, TNF-α was significantly ($P < 0.05$) increased in offspring born to dams fed high dietary soy isoflavones. Spleen of rats born to dams fed high dose of dietary soy isoflavones showed coagulative necrosis in white pulp. In conclusion, male offspring born to dams fed different levels of soy isoflavones showed marked immunosuppression after PHA stimulation. This effect was mediated through the reduced IFN-γ that interacts with the IL-12 production pathway.

1. Introduction

Progressive accumulation of endocrine disruptors in the environment has deteriorated the ecological balances in natural populations and affected human health [1]. Although estrogen hormone and estrogen like substances can promote both humoral and cell-mediated immune responses, there are a considerable number of reports that show the suppressing effect of estrogens on some cell-mediated immune responses [2,3]. Phytoestrogens are natural polyphenolic non-steroidal plant compounds with estrogen-like biological activities [4,5] and structurally are similar to mammalian estrogen 17β-estradiol) E2) [5]. The isoflavones genistein (GEN) and daidzein are among the most abundant phytoestrogens in human diets [6]. They can be classified as selective estrogen receptor modulators (SERMs) [7,8], where they have the ability to trigger estrogenic activity to act as agonist or antagonist [9,10] depending on the tissue, estrogen receptors (ERs) and concentration of circulating endogenous estrogens [10]. The interaction between isoflavones and nuclear estrogen receptors that activates estrogen response elements is called genomic signaling pathway [11]. Another more faster and rapid action of isoflavones is mediated through binding of membrane ERs [12]. Binding membrane ERs promotes a cascade of intracellular events that comprises activation of G-proteins, protein kinase, phospholipase, or adenylate cyclase activities [13]. Isoflavones can act as tyrosine kinase inhibitors [14]. Moreover, these compounds possess antioxidant activity [15] due to its polyphenolic nature [16]. Isoflavones exert myriad effects on different body systems and organs. They can affect immune system [2,17], reproductive system [4,18], nervous system [19], liver [6], bone [20] as well as their potential antioxidant effect and antidiabetic effect [21].

Interleukin 12 (IL-12) is an important immunoregulatory cytokine that is produced mainly by antigen-presenting cells. The expression of IL-12 during infection regulates innate responses and outlines the type

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of the adaptive immune responses to be triggered. IL-12 can provoke the production of interferon-γ (IFN-γ) and triggers $CD4^+$ T cells to differentiate into type 1 T helper (Th1) cells [22]. TNF-α has a crucial role in immune regulation by modulating lymphocyte proliferation and apoptosis, which is implicated in maintaining immune homeostasis and self-tolerance [23]. TNF-α activates cell inflammation, proliferation, survival and cell death depending on autocrine/paracrine signals, and on the cellular context [24,25].

In toxicity studies, endocrine-mediated effects have been reported in rat pups of dams treated with GEN during the gestational and/or lactation periods [26–28]. Moreover, most studies have focused on the effects of estrogenic pesticides and toxic substances on immune function [29,30]; less attention has been paid to the effects of naturally occurring phytoestrogens administrated during pregnancy on the immune system of the male's first generation. Therefore, the aim of this study was to examine the effects of maternal exposure to soy isoflavones on cellular immune response of male offspring through determination of the immune response to intradermal PHA injection, IL-12, IFN-γ and TNF-α levels and to investigate the histopathological changes in foot pad, spleen and thymus.

2. Materials and methods

2.1. Rats

Forty five adult (36 females and 9 males), Wister albino rats, weighing from 180 to 250 g, were housed in a plastic cage (3/cage) at Laboratory Animal House, Faculty of Veterinary Medicine, Suez Canal University, Egypt. They were maintained under standard natural day light with a temperature of 25° C (± 1° C) and allowed to diet and water *ad libitum*. The animals were treated according to ethical guidelines described by Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

2.2. Monitoring estrous cycle and breeding

The estrous cycle of the rats was checked daily by cytological examination of vaginal smear to determine the females with regular cycles. Vaginal smears were obtained, processed and evaluated according to Ebeid et al. [31]. A mature male was presented with three proestrus females and mating was confirmed by the presence of spermatozoa in the vaginal smears or the occurrence of vaginal plug and this was considered the pregnancy Day zero [32].

2.3. Isoflavones analysis and administration

Isoflavones were extracted, detected and quantified from the diet using high performance liquid chromatography (HPLC) according to Thiagarajan et al. [33]. Briefly, soy isoflavones were extracted from the experimental diet by mixing 1 g of diet with 20 mL of solution of 0.1 mol/L HCL and 80 mL of methanol, and then sonication of mixture was performed for 20 min and left at room temperature for 2 h. Filtration using Whatman filter paper (Clifton, New Jersey) was performed. The obtained filtrate was subjected to centrifugation at 10,000 rpm. The obtained supernatants were quantified for isoflavones contents. Isoflavones were quantified by comparison with genistein (Applichem GmbH Co., Germany) and daidzein, (Fullcco Co., Japan) with HPLC standards.

The female rats at Day zero of pregnancy were allocated to three groups. The first group (n = 12) were fed a control diet (soy isoflavones free). The second group (n = 12) were fed low dose of soy isoflavones (6.5%). The third group (n = 12) were fed high dose of soy isoflavones (26%). All diets were formulated to fulfill all the nutritional requirements of pregnant rats [34]. The percentages of soy isoflavones in both treated groups covered the level of 20–50 g of daily soy as a source of phytoestrogens that consumed by Asian population [35]. Experimental diets were offered from Day zero of pregnancy to the Day of birth to the dams. After parturition, male offspring were selected and were given control diet up to Day 50 after birth which was defined as post natal day 50 (PND 50).

2.4. Cell mediated immune response in male offspring

Offspring's cell mediated immune response was carried out by injection of 0.1 mL of 10% PHA (Sigma L 9017, St. Louis, MO, USA) in left foot pad of each male in all experimental groups at PND 49. The right foot pad of the same rat was injected with 0.1 mL of PBS as a control. After 24 h (PND 50), thickness of dorso-ventral and lateral aspects of left footpad at point of injection was measured by using a manual micrometer [36]. The injections and measurements were made by the same person to reduce the error.

2.5. Determination of intumesce index

The ankle circumference was calculated according to = 2π [*sqrt* $(a^2 + b^2/2)$], where [a] is the dorso-lateral diameter and [b] is the dorso ventral diameter [37,38]. This was followed by calculation of intumesce index [39]. Intumesce Index = (measured ankle size – primary ankle size)/primary ankle size.

2.6. Determination of serum IL-12, IFN-γ and TNF-α

Blood samples were collected at the end of experimental period (PND 50). Serum was separated and kept at −20 °C until analysis. Serum IL-12 was measured using rat IL-12/P70 sandwich ELISA kit (CUSABIO, China). Serum IFN-γ was measured using rat IFN-γ sandwich ELISA kit (R&D systems, China). Serum TNF-α was measured using rat TNF-α enzyme linked immunosorbent assay sandwich ELISA kit (IBL Co., Japan) according to manufacturer instructions.

2.7. Spleen and thymus relative weights

At PND 50, male offspring were scarified and the relative weight of spleen and thymus was calculated in relation to body weight [40,41].

2.8. Histopathology

PHA stimulated foot pads, spleen and thymus of males were fixed in 10% formalin buffer saline. They were gradually dehydrated then embedded in paraffin wax. Several 5-μm sections were cut then stained with hematoxylin and eosin (H&E) for histopathological examination [42].

2.9. Statistical analysis

The results were presented as the mean ± standard error of mean (SEM). Statistically significant differences between groups were calculated using one way analysis of variance (ANOVA) followed by Duncan's post hoc multiple comparison test (SPSS software, version 16.0; SPSS Inc., Chicago, IL, USA). Dose-response to soy supplementation on the immune parameters was evaluated and soy-supplemented groups were compared with the control group for linear and quadratic contrasts using the generalized linear models procedure of SAS 9.3 (SAS Institute Inc., Cary, North Carolina, USA). The criterion for significance was set at $P < 0.05$.

3. Results

3.1. The number of male offspring

The number of male pups/dam was 3.0 ± 0.6 in control group, while, it was 3.2 ± 0.7 and 2.8 ± 0.4 in dams received low and high